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Anticancer activities of mushroom polysaccharides on chemically-induced colorectal cancer in rats

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ABSTRACT

Polysaccharides from mushroom *Pleurotus sajor-caju* (PS1) and *Lactuca Sativa* (PS2) were isolated and purified (22.40 and 26.80g/100g respectively). Cytotoxic activities of PS1 and PS2 were examined *In vitro* using colon (HCT 116), liver (HEPG2), cervical (HELA) and breast (MCF7) carcinoma cell lines. The present results indicated that these polysaccharides (PS1 and PS2) have more inhibitory effects on HCT-116 than the other carcinoma cell line (HEPG2, HELA and MCF7). Polysaccharides (PS1 and PS2)-treated HCT-116 cells showed a high percentage of cell death, indicating promising anti-tumorigenic properties, and demonstrate their direct effect on colon cancer cell proliferation. Evaluation of the two polysaccharides (PS1and PS2) was carried out through determination of the tumor markers (CEA and C19.9) in cancer rat groups treated with either PS1 or PS2 compared versus carcinogenic control rats. Polysaccharides (PS2) administration caused higher inhibitory effect on chemically-induced colorectal cancer in rats through the histological examination and a marked reduction in the levels of ALP and ALT more than that of PS1 in serum of rats. It can be concluded that polysaccharides (PS1 and PS2) are more effective for inhibition of chemically induced colorectal cancer in rats.

INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Jemal *et al.*, 2011). WHO (2006) estimates that 84 million people will die of cancer between 2005 and 2015. Chemotherapy is one of the most frequently used therapeutic modalities for the treatment of cancer, but it does not achieve a satisfactory therapeutic result if it is used alone. The colorectal cancer is the most malignant tumor with very high morbidity and mortality rates, and poor prognosis (Li *et al.*, 2009; Shengtao *et al.*, 2012). Colon cancer is considered a preventable disease (Giovannucci *et al.*, 2002). However, it seems to be that, there is no decline in the incidence of colon cancer, and many of the risk factors associated with colon cancer prevail. Diet-based strategies hold promise for both prevention and treatment of colon cancer (Milner *et al.*, 2001). In this regard, plant-derived diets containing phytochemicals and/or polysaccharides could be used in preventive strategies to reduce the risk and inhibit or retard the development of colon cancer (Raju *et al.*, 2004). Considering the continuous need for effective anticancer agents, medicinal plants might be an inexhaustible source of anticancer drugs in terms of both variety and mechanism of action. Epidemiological investigations indicated that diets with high fruits and vegetables provide a mean of cancer chemoprevention due to their phytochemical constituents (Reynertson *et al.*, 2011). In recent years, much attention has been focused on polysaccharides isolated from natural sources such as bacteria, fungi, algae and plants (Jwanny *et al.*, 2009; Sun, 2011). Polysaccharides from natural sources are found to be effective, non-toxic substances with wide variety of biological activities, and have attracted lots of attention in the biochemical and medical areas (Ooi and Liu, 2000). Experimental studies demonstrated that many naturally occurring agents and plant extracts have anticancer potential in a variety of bioassays systems and animal models, having relevance to human diseases (Sun, 2011). A chemical modification of the polysaccharides extract derived from Leucaena leucocephala seeds may acts as a potent anti-inflammatory agent and its sulphated derivative may acts as an inducer of macrophage functions against pathogens (Amira *et al.*, 2007).

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Lactuca sativa (Lettuce, compositae) is a well-known vegetable as well as a medicinal plant is consumed globally. Traditionally it is famous for its use as folk remedy for inflammation, pain, stomach problems including indigestion and for lack of appetite. Considerable pharmacological studies have been conducted to evaluate therapeutic significance of the crude extracts of *L. sativa*. Edible mushrooms have been reported to generate beneficial effects for health and treatment of some diseases through their immunomodulatory and antineoplastic properties (Finimundya *et al.,* 2013). Mushroom is a simple form of life known as fungus. Mushroom proteins contains essential amino acids required for human body, has no cholesterol content, easily digested and considered intermediate between animals and vegetables constituents (Roshita *et al.,* 2012).

Mushroom polysaccharides, such as (1→3), (1→6)-b glucans, 14 (1→3)-a-glucans act as immunomodulating and anti-tumor materials. Water-soluble polysaccharides have been isolated from the fruit bodies of P. sajor-caju. was characterized and reported (Pramanik *et al.,* 2007).Water polysaccharide extracts have been shown to prevent tumor growth in mice especially the high-molecular-weight (Cheung *et al.,* 2002; Jwanny *et al.,* 2002).

The mushroom *Pleurotus sajor-caju* fruits and *L. sativa* leaves are edible and are used as condiments and as Ayurvedic medicine in different countries Plant extracts constituents have been found to have anticarcinogenic potency in different settings. This extract has been evaluated in the *Ehrlich ascites* carcinoma model in BALB/c mice, where it effected 70% inhibition of tumor cell growth compared with controls (Sur *et al.,* 2001; Hibasami *et al.,* 2003). Some investigators provided evidence that polysaccharides consumption results in protection against chemically induced large bowel cancer (Jwanny *et al.,* 2002; Chena *et al.,* 2012).

Bioactive compounds have been isolated in samples collected from different region of Egypt. However, there are many active polysaccharides in different organ of plant (Moharib, 2006; Sun, 2011). Abd el Monem *et al.,* 2013 revealed that the crude polysaccharide in some plant has obvious hydroxyl radical activity (Moharib and Awad, 2012).

Evidence from various studies suggest that metabolites derived from plants may possess pro-apoptotic properties and have great potential for possible applications in cancer prevention (Prasanna *et al.,* 2009; Choedon *et al.,* 2010). The antitumor activities of polysaccharides were evaluated in an *In vitro* studies but little research were published on the antitumor activity of polysaccharide isolated from plant origin in vivo.

In view of this, the present investigation aimed to, extract, isolate and purify polysaccharides from *L. sativa* (PS2) and from *P. sajor-caju* (PS1). The chemical compositions of the purified polysaccharides (PS1 and PS2) were determined. The inhibitory effect of these polysaccharides *In vitro* was studied as a new cancer chemo- preventative and investigation of their anticancer properties was done in vivo using chemically induced colon cancer in rats. The ultimate goal is to use those derivatives as alternatives of polysaccharide-protein complex in health food industries and to provide potential cancer chemo-preventive and/or anticancer properties for high risk population.

MATERIALS AND METHODS

Carcinogenic material used in this study was 1, 2 dimethylhydrazine dihydrochloride 99+% (DMH) and was obtained from Sigma-Aldrich® chemie, Gmbh, Riedstr. 2, D-89555 Steinheim, Germany. D-glucose,D-galactose, D-mannose, D-xylose, L-arabinose, L-fucose and L-rhamnose used as standards were purchased from Sigma Chemical Co. Fresh Leaves of *L. sativa* were obtained from an Egyptian local market and mushroom *P. sajor-caju* fruits were obtained from Agriculture research center, Giza, Egypt.

The mushroom *P. sajor-caju* fruits and *L. sativa* leaves were cut into small pieces then they were dried in an oven at 50ºC till constant weight. Finally, the dried Materials were ground in a food grinder (mincer) to a very fine powder, sifted through a 16-mesh sieve, packed in bags, and stored at room temperature till used. The fats of mushroom *P. sajor-caju* fruits and *L. sativa* leaves were removed using petroleum ether (boiling range 60-80 °C) at 80 °C. The proteins were removed using Rashad *et al.,* method (2000).

Extraction and purification of polysaccharides

The dried mushroom *P. sajor-caju* fruits and *L. sativa* leaves, previously prepared were soaked with water and homogenized using homogenizer (Mechanika precyzyjna warszawa model MPW-309, Poland) and used for extraction of its polysaccharides (PS1 and PS2 respectively) as described by Staub, (1965) and Chihara *et al.,* (1970) using hot water bath (80°C) for 18 hours and cooled at room temperature. Five volumes of ethanol were added to precipitate crude polysaccharides. The precipitates was recovered by centrifugation and washed successively with ethanol, followed by drying at 50 °C, yielding crude polysaccharide.

The crude polysaccharides were dissolved in water (100 ml) and deproteinized using trichloroacetic acid (TCA) method, and the deproteinated polysaccharide was obtained. Total carbohydrate and protein of these deproteinized and defatted polysaccharides were determined (Dubois *et al.,* 1956; Lowery *et al.,* 1951). Monosaccharides contents of polysaccharides were measured using a paper chromatographic technique (Wilson, 1959; Jwanny and Hussein, 1976).

In vitro studies

Cytotoxicity test of the two polysaccharides (PS1 and PS2) were done *In vitro* using different human cancer cell line particularly those of colon (HCT 116), liver (HEPG2), cervical (HELA) and breast (MCF7) carcinoma cell lines. Measurements of potential cytotoxicity of the samples were assayed by sulforhodamine B (SRB) according to the method described by Skehan *et al.,* (1990).

In vivo studies

Induction of colorectal cancer in rats was done using 1,2 dimethyl hydrazine (DMH) according to method of Cheng *et al.*, (2003) and Jwanny *et al.*, (2009).

Animals

Thirty five male albino rats, 8 weeks of age, weighing about 140 - 150g were purchased from the National Research Center for biological products. The rats were divided into five groups (7 rats/group) and housed in a wire screen cage. The rats had free access to fed commercial diet and tap water. The animal room was controlled (25 ± 1 ºC) and had a 12-hour light-dark cycle and humidity at 60 ± 5%. The rats were acclimatized for a period of two weeks before the experiments began. Three groups of rats (C) were administrated for 5 weeks (twice /week) subcutaneous injections of 1,2-dimethyl-hydrazine (DMH) at a dose of 40 mg / kg body weight (Cheng *et al.*, 2003; Jwanny *et al.*, 2009). The first group (C) was maintained without any treatment over experimental period (16 weeks) and used as carcinogenic control group (C). The other 2 groups of rat administrated DMH for 5 weeks (twice /week) were treated (C/PS1 and C/PS2) with oral dose (100 mg / kg body wt / day) of each polysaccharides (PS1 and PS2 respectively) from week 6 till the end of the experimental period (16 weeks). The other two groups (PS1/C and PS2/C) of rats was treated daily with oral dose (100 mg / kg body wt / day) of each polysaccharides (PS1 and PS2 respectively) for a period of 5 weeks (twice / week) and then they were administrated with DMH at a dose of 40 mg/kg body weight for 5 weeks (twice/week). The experimental protocol was done according to the methods of George *et al.*, (2011).

Serological markers

After 16 weeks, blood samples were drawn from 7 rats per each group separately using capillary tubes, centrifuged at 4000 xg for 10 min. Separated sera were used for different biochemical analysis. Liver and colon were removed and used for pathological examinations.

Alkaline phosphatase (ALP) level (IU/L) was carried out referring the DGKC indications, Germany (1972). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured (U/L) according to the method of Reitman and Frankel, (1957), using kits of QCA, Spain. Gamma glutamyl transferase (GGT) was carried out according to the kinetic colorimetric method of Szasz, (1969), using Biodignostic kits, Egypt. Total protein (gm/dL) was estimated according to the method of Bradford, (1976) using Biodignostic kits, Egypt. Total lipid (mg /dL) was estimated according to the method of Knight, *et al.*, (1972) using Biodignostic kits, Egypt. Phospholipid (mg/ dL) was estimated according to the method of Takayama *et al.*, (1977) using kits of Biodignostic, Egypt. Phospholipid phosphorous (PP, mg/ dL) was estimated according to the method of Connerty *et al.*, (1961). Glutatione reductase (GR) (U/L) was estimated according to the method of Goldberg and Spooner, (1992) using kits of Biodignostic, Egypt. Lipid peroxidase (LPx) was estimated according to the method of Ohkawa *et al.*, (1979). Quantitative determination of CEA (ng/ml) was performed with commercially available Enzyme Immunoassay Kit (Bio Check, Inc. catalog number: BC-1011), (Uotila *et al.*, 1981). CA 19.9 was performed (Pilo et al. 1996) with commercially available Enzyme Immunoassay Kit (Invitrogen, catalog number: 99-0070).

Statistical Analysis

All statements of significance were based on a probability of $P < 0.05$. Data from the molecular biology studies were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System (SAS, 1986) followed by the Scheffé-test to assess significant differences among groups.

Histology

Histological assessments of liver and colon tissues were carried out according to Scheuer and Chalk (1986) using Hematoxyline and Eosin (H&E) staining technique.

Results

Polysaccharides were extracted and partially purified from *P.sajor-caju* fruits (PS1) and *L. sativa* leaves (PS2) by hot water and precipitation by 5volumes of ethyl alcohol with yields of 9.20% and 12.40% respectively. These results showed that the amount of polysaccharides in PS2 was higher than that of PS2. Total carbohydrate contents in PS1 and PS2 were estimated (96.4 and 98.2% respectively) using the phenol–sulfuric acid method and small amount of protein were observed. The molecular weight of PS1 and PS2 were estimated to be 240 and 250 kDa respectively according to the calibration curve prepared using dextrans.

Paper chromatographic analysis revealed that there are different values of individual monomers in both polysaccharides obtained (PS1 and PS2) such as rhamnose, arabinose, xylose, mannose, galactose, and glucose. The chromatographic analysis of PS1 indicated that the major component were glucose (36.60%), galactose (32.40%) and mannose (30.80%). Paper chromatographic analysis of hydrolyzed PS2 composed of glucose (34.18%), galactose (26.53%), mannose (24.4%), arabinose (10.52%), xylose (1.16%) and rhamnose (2.84%). The results indicated that glucose, galactose and mannose were the predominant monosaccharide in PS1and PS2. These polysaccharides may have biological and physiological importance and has different effects on some diseases and chemically induced cancer.

***In vitro* studies**

Measurement of potential cytotoxicity

The main objective of this study was to evaluate the potential efficacy of the two polysaccharides obtained from both *P.sajor caju* and *L. sativa* (PS1 and PS2 respectively) against colon cancer *In vitro* and in vivo. The present study was carried out to screen the compounds that were extracted and purified using *In vitro* cytotoxicity test to identify activity of the prepared

compounds (PS1 and PS2) in growth inhibition of four different tumor cell lines colon (HCT-116), liver (HEPG2), cervical (HELA) and Breast (MCF7). Results (Fig.1, 2) showed that PS1and PS2 were more effective in inhibition of HCT-116 but lower effective against liver (HEPG2), cervical (HELA) and Breast (MCF7) cancer cells. Cytotoxic activities of PS1 and PS2 were examined using liver (HEPG2), colon (HCT 116), breast (MCF7) and cervical (HELA) cancer cells *In vitro*. Results (fig. 2) showed that PS2 was more effective in inhibition of both HCT-116 and HepG2 but not effective against breast (MCF7) and cervical (HELA) cancer cells *In vitro*. Using PS1 exhibited more effectiveness on liver (HEPG2), colon (HCT 116) but less on breast (MCF7) and cervical (HELA) cancer cells (fig. 1).

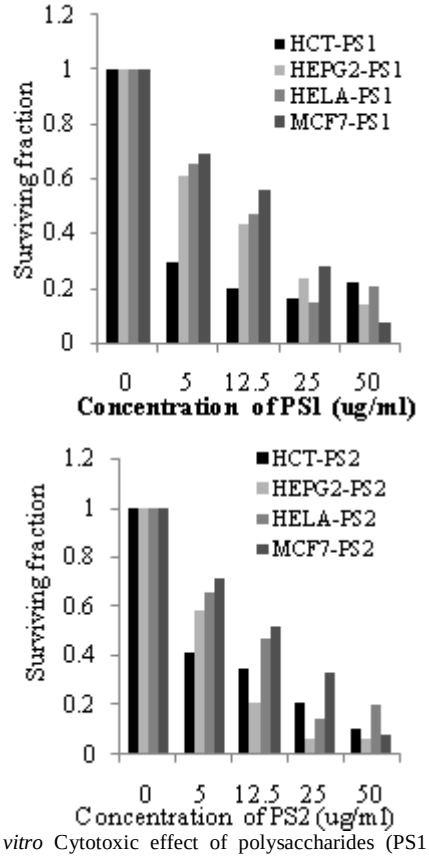

Fig.1, 2: *In vitro* Cytotoxic effect of polysaccharides (PS1 and PS2) on different human cancer cell line particularly liver (HEPG2), colon (HCT 116), breast (MCF7) and cervical (HELA) carcinoma cell line.

Similar results were reported when examined glucan and polysaccharides extracted from *P. ostreatus* on colon cell line *In vitro*. So it can be observed that polysaccharides inhibit cell proliferation in HCT-116 human colon cancer cell lines. That could arrest the cell cycle and generate apoptosis, which explains the *In vitro* anti-proliferative effect of polysaccharides.

Results in Figure (3), illustrate the dose response (IC50) of PS1 and PS2 on HCT116 cells. The present results also, showed the growth inhibitory effect of PS1 and PS2 on HCT116 cell lines. The data showed that *L. sativa* polysaccharides (PS2) have a cytotoxic activity against the colon (HCT116) cancers than other cell line. This indicated that **PS1 and** PS2 have anticancer activity against colon carcinoma. The PS1 and PS2 reduced the survival fraction to 50% (kills 50% of the cancer cells) where less than 5µg of PS1 and PS2 killed 50 % of the cancer cells particularly colon cancer cell lines (IC50).

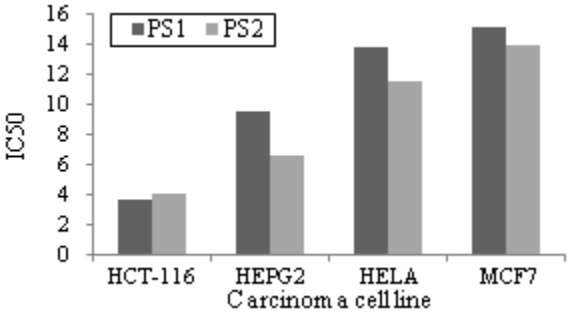

Fig. 3: IC50 of PS1 and PS2 on HCT116 cells.

The present study was carried out to screen the compounds that extracted and purified using *In vitro* cytotoxicity test to identify activity of the extracred compounds (PS1 and PS2) in growth inhibition of different tumor cell lines i.e. colon (HCT 116), liver (HEPG2), cervical (HELA) and Breast (MCF7) carcinoma cell line.

Previous study showed that supplementation of *T. foenumgraecum* in the diet inhibits colon carcinogenesis, by modulating the activities of beta-glucuronidase and mucinase.

The present study establishes that polysaccharide of *P. sajor caj* (PS1) has appreciable anti-cancer activity greater than that of *L. sativa* (PS2). However, based on the published studies, administration of *P. sajor caj* and *L. sativa* to man is simple, since they are used as common dietary constituents in many parts of the world.

In vivo study

Biochemistry

The present results in fig (4 A, B) and table (1) indicated higher significant increases in the level of ALP, ALT, GGT and AST in sera of rats administered DMH (group C). Higher significant decreases were observed in the levels of ALP, ALT, GGT and AST in sera of rats administered PS1 and PS2 compared to those administered DMH (group C) (fig. 4 A,B). Results also showed highly significant decreases in the levels of ALP (%) and ALT %) in PS1/C rat group compared to group C. Insignificant changes were observed in AST.

A marked reduction were observed in the levels of ALP and ALT (%), in sera of rat groups (administered pre- treatment of PS1/C and PS2/C before inducing colon cancer) compared to group C. AST level was deceased significantly in sera of rat groups administered PS2 (C/PS2 and PS2/C) more than those administered PS1 (C/PS1 and PS1/C). The present results showed high significant decreases in the levels of GGT (U/L) in sera of rat groups administered PS1 (PS1/C and CPS1) and PS2 (PS2/C and C/PS2) compared to group C (fig 4A). Similar results were reported by other using different type of polysaccharides.

Table. 1: Biochemical parameters in sera of experimental rats.

Parameters	Control	C/PS1	C/PS2	PS1/C	PS2/C
ALP(IU/L)	270.1 ± 2.14	176.45 ± 1.97	144.04 ± 1.30	113.1 ± 0.95	105.2 ± 0.90
ALT (U/ml)	40.4 ± 0.97	7.3 ± 0.38	7.52 ± 0.77	5.3 ± 0.88	4.4 ± 0.63
AST (U/ml)	45.63 ± 1.37	14.60 ± 0.75	12.64 ± 1052	16.94 ± 0.61	10.03 ± 0.39
GGT (U/L)	160.4 ± 1.35	86.13 ± 0.64	48.22 ± 0.44	46.8 ± 0.26	42.4 ± 0.40
Total protein (gm/dl)	4.06 ± 0.20	6.95 ± 0.23	6.16 ± 0.24	6.12 ± 0.14	6.04 ± 0.33
Total lipid (mg/dl)	473.6 ± 0.97	290.00 ± 1.86	339.67 ± 1.18	302.20 ± 2.27	299.80 ± 1.53
LPX (nmol/ml)	3.03 ± 0.34	2.40 ± 0.25	1.61 ± 0.26	2.2 ± 0.10	1.39 ± 0.33
GR (U/l)	631.00 ± 2.19	875.17 ± 2.36	884.40 ± 3.56	990.71 ± 2.41	996.86 ± 3.72
PP (mg/dl)	43.82 ± 0.41	79.17 ± 2.40	46.56 ± 2.31	76.14 ± 2.38	50.54 ± 2.26
Phospholipid (mg/dl)	10.96 ± 0.20	6.39 ± 0.60	5.93 ± 0.64	7.14 ± 0.65	7.45 ± 0.28

(Mean value ± SE of 7 rats / group)

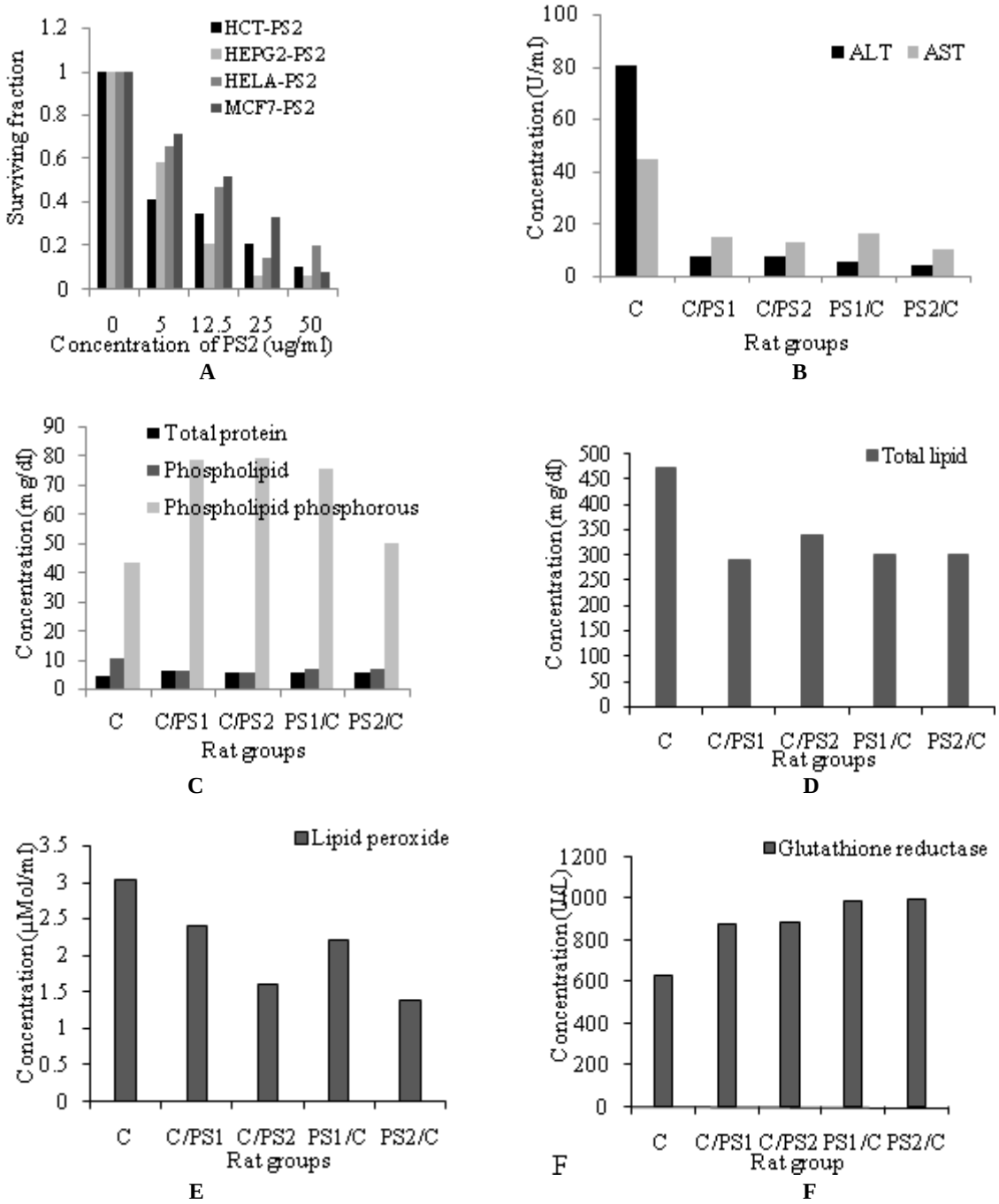

Fig. 4: Biochemical parameters in sera of experimental rats. (A) ALP, GGT, (B) ALT, AST, (C) Total protein, Phospholipid and Phospholipid phosphorous, (D) Total lipids, (E) Lipid peroxide and (F) Glutathione reductase. Data was presented as mean value ± SE of (7 rats / group).

High significant increases were observed in the levels of total protein in sera of rats administered PS1 (PS1/C and CPS1) and PS2(PS2/C and C/PS2) compared to group C (Fig. 4C). High significant increases were observed in the levels of total protein in sera of rats administered PS1 (PS1/C) and PS2(PS2/C) more than those of rats administered PS1(C/PS1) and PS2(C/PS2).

The present results also showed high significant decreases in the levels of Phospholipids and PP in sera of rats administered PS1 (PS1/C and C/PS1) and PS2 (PS2/C and C/PS2) compared to group C. A marked reduction in the levels of phospholipids in sera of rats administered PS1(C/PS1) and PS2(C/PS2) more than those of PS1(PS1/C) and PS2(PS2/C) as shown in (fig.4C). On contrast, significant increases in the levels of PP in sera of rats administered PS1(PS1/C and CPS1) and PS2(PS2/C and C/PS2) compared to group C. High significant increases in the levels of PP in sera of rats administered PS1(C/PS1) and PS2(C/PS2) more than those of PS1(PS1/C) and PS2(PS2/C) and compared to group C. The results exhibited highly significant increases (fig.4E) in the levels of GR in sera of rats administered PS1 (PS1/C and CPS1) and PS2 (PS2/C and C/PS2) compared to those administered DMH (control group C). The highest significant increases in the levels of GR were observed in sera of rats administered PS1 (PS1/C) and PS2 (PS2/C) more than those of PS1(C/PS1) and PS2(C/PS2).On contrast, significant decreases in the levels of lipid peroxide (LPX) in sera of rats administered PS1 (PS1/C and CPS1) and PS2 (PS2/C and C/PS2) compared to those administered DMH (control group C). The high significant decreases in the levels of LPX were observed in sera of rats administered PS2 (PS2/C and C/PS2) more than those of PS1(C/PS1 and PS1/C). (fig.4E).

The present data showed that the levels of CEA was decreased in the sera of all the studied rat groups compared to group C. Insignificant difference was observed in the levels of CA/19.9 (Table 3, fig. 5).

Table. 2: CEA and C19.9 levels in sera of experimental rats.

Tumor markers	C	C/PS1	C/PS2	PS1/C	PS2/C
CEA (ng/ml)	4.14 ± 0.14	4.00 ± 0.20	3.30 ± 0.17	1.68 ± 0.56	0.40 ± 0.11
CA/19.9 (ng/ml)	1.50 ± 0.60	1.40 ± 0.54	1.11 ± 0.38	1.01 ± 0.37	0.81 ± 0.49

(Mean value ± SE of 7 rats / group)

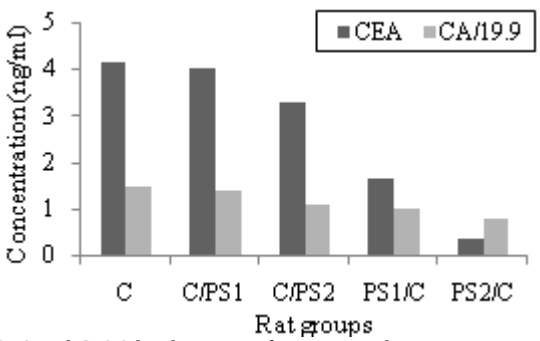

Fig. 5: CEA and C19.9 levels in sera of experimental rats. Data was presented as mean value ± SE of (7 rats / group).

Histology

Examined sections of rat colon from carcinogenic group (C) revealed necrosis of each of the intestinal villi (Fig. 6a) and lymphocytes (in submucosa) and glandular epithelium (small arrow) associated with preglandular (big arrow) fibrosis (Fig. 6b). Treatment with PS2/C causes some improvement in these histological changes (Fig. 6d). Colon from PS1/C group showed necrosis of both the glands (small arrow) and of lymphocytes (big arrow) of submucosa (Fig. 6c).

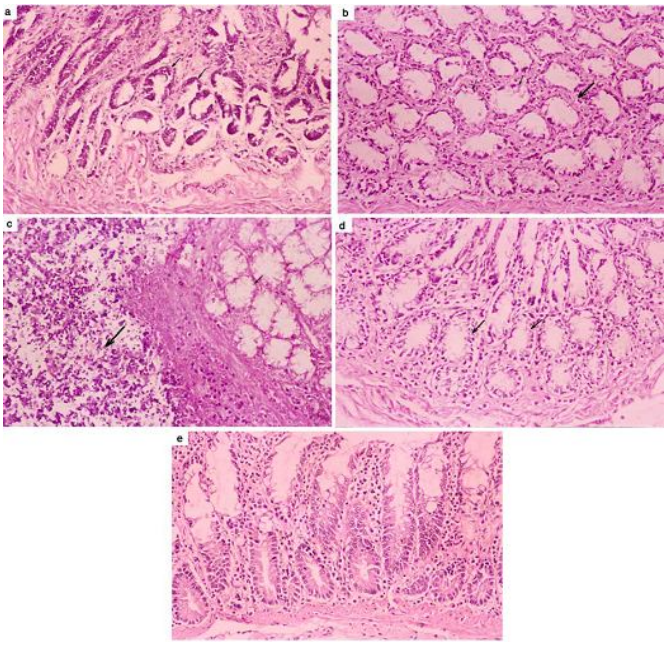

Fig. 6: Sections of colon of cancer rats (group C) (a,b) and treated rats with either PS1 (c,d) or PS2 (e).

DISCUSSION

Non-cellulosic b-glucans are now recognized as potent immunological activators, and are used clinically in China and Japan. These b-glucans consist of a backbone of glucose residues linked by b-(1/3) -glycosidic bonds, often with attached side-chain glucose residues joined by b-(1/6) linkages. Several investigators suggests that b-glucans and other polysaccharides are effective in treating diseases, cancer, and range of microbial infections, hypercholesterolaemia, and diabetes (Jiezhong and Robert, 2007).

The present work was done to investigate the anticancer activity of polysaccharides (PS1 and PS2) on the chemically induced colon cancer. The colon cancer was induced by intraperitoneal injection of a dose of 1,2-dimethyl-hydrazine (DMH) at a dose of 40 mg/kg body weight (twice a week for 5 weeks (Rasmy *et al.,* 2011). Previous studies indicated that longer periods (twice a week for 8 or 12 weeks) of DMH treatment led to the development of colon carcinoma (Cheng *et al.,* 2003). *L. sativa* has also shown an overall stimulatory effect on the specific as well as non-specific immune functions in mice (Bin-Hafeez *et al.,* 2003). The main chemical constituents of *L. sativa* are polysaccharides (Amin *et al.,* 2005). Mushroom has also shown an

overall stimulatory effect on the specific as well as non-specific immune functions in mice (Bin-Hafeez *et al.,* 2003). The PS1 and PS2 were tested for cytotoxic activity against the liver (HEPG2), colon (HCT 116), breast (MCF7) and cervical (HELA) carcinoma cell line *In vitro*. The results indicated that PS1 and PS2 have anticancer effect against all particularly colon and liver carcinoma. PS1 and PS2 reduces the survival fraction to 50%, it means that PS1 and PS2 kill 50% of the colon cancer cells lines. Lajvardi *et al.,* 1993; Campbell *et al.,* 1997; Suresh et al. 2012 demonstrated that the different effects of polysaccharides were dependant on their type (dose, structure, soluble and insoluble) and on the duration of the experiment.

The histopathological studies indicated that the treatments with PS1 and PS2 improved the histology in rats received the carcinogen DMH firstly for 5 weeks, then treated with the PS1 and PS2 for the remaining period of the experiment (C/PS1 and C/PS2). Chromatographic analysis revealed that there were different monosaccharides of both PS1 and PS2 such as mannose, galactose, glucose rhamnose, arabinose, xylose. Similar results were obtained by other investigators by using cabbage, sugar beet, *Jerusalem artichoke* and rhubarb (Goel, *et al.,* 1997; Jwanny *et al.,* 2009; Wu et al. 2012).

Serum transaminases are considered to be sensitive indicators of liver injury. The hepatic damage was indicated by increases in ALT and AST levels. These changes result from the leakage of enzymes from the hepatocytes. The increases of transaminases levels by carcinogenic are consistent with previous reports (Visen *et al.,* 1993). The elevation of sera ALT and AST was found in rats of group C more than that in PS1 and PS2 treated group (C/PS1 and C/PS2). Liver damage induced by chronic treatment that leads to liver cell necrosis and consequently elevated levels of serum transaminases.

In the present study, The results showed that, the Alanine aminotransferase enzyme (ALT), and Aspartate aminotransferase enzyme (AST) levels were significantly higher in sera of rats received the carcinogenic material DMH for all period of the experiment group (C) than in rats received the carcinogenic material firstly for 5 weeks then treated with the PS1 and PS2 for the remaining period of the experiment (C/PS1 and C/PS2). The value of ALT and AST activities in the sera of rats (PS1/C and PS2/C) reflected their improvements of liver function. On contrast the values of ALT and AST of rats administered DMH reflected their abnormal liver function. The results of the present study indicated that, the PS1 and PS2 lead to improve the ALT and AST levels. These results are consistent to other studies made by Visen *et al.,* 1993; Muqbil, *et al.,* 2005; Jwanny *et al.,* 2009; Wu et al. 2012).

(Khan *et al.,* 2005), studied the inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*. They reported the chemopreventive effect of *Nigella sativa* against ferric nitrilotriacetate induced renal oxidative stress, hyperproliferative response and renal carcinogenesis. It also enhanced DEN (N-diethylnitrosamine)-initiated renal carcinogenesis by increasing the percentage incidence of tumours. Treatment of rats orally with Nigella sativa (50 and 100 mg/kg body weight) resulted in significant decrease in lipid peroxidation, xanthine oxidase, H_2O_2 generation and incidence of tumours. The researchers suggest that, *Nigella sativa* is a potent chemopreventive agent and suppresses oxidative stress, hyperproliferative response and renal carcinogenesis in rats.

(Suresh *et al.,* 2012) and (Ali *et al.,* 2004) studied the hepatoprotective effects they induced liver damage in rats. The degree of protection was evaluated by determining the marker enzymes (AST, ALT and ALP) and total proteins. Further, the effects on lipid peroxidation and glutathione, Lipd peroxide and glutathione reductase (GR) were estimated to evaluate antioxidant activity (Suresh *et al.,* 2012) concluded that, the hepatoprotective effects of *Nigella sativa* against oxidative damage may be due to its antioxidant and free radical-scavenging activity.

(Muqbil *et al.,* 2005), studied the enhancement of prooxidant effect of 7,12-dimethylbenzen in rat. Biochemical measurements were carried out on sera of control and treated animals. Restraint stress was found to have marked effect on DMBA induced alteration of liver function as revealed by the increase in tissue marker enzymes via AST,ALT, ALP and lactate dehydrogenase (LDH) with a significant further decrease in antioxidant enzymes superoxide dismutase (SOD), glutathione-*S*-transferase, GR as compared to controls.

In the present study, the results showed that, the ALP activity were significantly higher in rats of group C received the carcinogenic material DMH for all period of the experiment than that in rats received the carcinogenic material firstly for 5 weeks then treated with PS1 and PS2 for the remaining period of the experiment, than those of rats treated with the PS for all period of the experiment. These results are consistent to other studies made by (Muqbil *et al.,* 2005, and Rasmy *et al.,* 2011) when using different materials.

In the present study, the results showed that, GR concentrations in the rat's liver tissue were significantly higher in rats treated with the PS1 and PS2 for all period of the experiment (C), and rats received the carcinogenic material firstly for 5 weeks then treated with PS1 and PS2, than in rats received the carcinogenic material DMH (C). The results of the present study indicated that, PS1 and PS2 tend to improve the GR concentrations in the rat tissues. These results are consistent to other studies reported by other investigators (Khan *et al.,* 2005 and Muqbil *et al.,* 2005).

Regarding, CEA and CA-19.9, they showed marked decrease in the groups treated with PS1 and PS2 before induction of colon cancer more than that decrease shown in the groups that treated with these PS after induction of tumor and both groups are markedly decreased compared to group C that is indicating that the role of treatment with theses polysaccharides should be considered (Abd el Monem *et al.,* 2013).

The present study was carried out to screen the compounds that were extracted and purified using *In vitro* cytotoxicity test to identify activity of the prepared compounds (PS1 and PS2) in growth inhibition of different tumor cell lines

(HEPG2, HCT 116, MCF7 and HELA) *In vitro*. Similar results were found by lavi *et al.,* 2006, when examined glucan extracted from *P. ostreatus* on colon cell line *In vitro* (Abd el Monem *et al.,* 2013). So it can be observed that polysaccharides PS1 and PS2 inhibit cell proliferation in HCT-116 human colon cancer cell lines. That could arrest the cell cycle and generate apoptosis, which explains the *In vitro* anti-proliferative effect of polysaccharides (Chen and Chang, 2004 and Jwanny *et al.,* 2009). Similar results were reported by other investigators (Bao *et al.,* 2002 when using different type of polysaccharides.

Raju *et al.,* 2004, observed a significant inhibition of the initiation and development of colon cancer when fenugreek was given during post-initiation or promotion stage. They concluded that fenugreek would be effective not only in preventing the appearance of ACF but plausibly also in retarding the growth and progression of large ACF, including those of the intermediate and advanced type. This aspect is very important considering that a large portion of the population at risk for colon cancer is characterized by the presence of polyps and large ACF in their colons (Bird and Good, 2000 and Takayama *et al.,* 2001).

The present study establishes that polysaccharide of *P. sajor caju* and *L. sativa* (PS1 and PS2 respectively) have appreciable anti-cancer activity. However, based on the published studies, administration of *P. sajor caju* and *L. sativa* by man is simple, since, they are used as common dietary constituents in many parts of the world.

CONCLUSION

It is known that most drugs isolated for cancer therapy are not cancer specific and, therefore, may be highly toxic to normal tissues, leading to serious adverse effects. Mushroom extracts might be considered alternative sources for adjuvant cancer therapy, as they have no adverse effects, activate the cells of the immune system, and reduce free radicals. Further studies, however, including the isolation and chemical characterization of the major compounds that contribute to the promotion of the immune system and to the inhibition of carcinogenesis, are needed and may generate new targets for therapy. Moreover, the present study establishes that polysaccharides of *L. sativa* leaves (PS2) and of *P.sajor-caju* (PS1) have appreciable anti-cancer activity and may be improve health.

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